Immunity
Letters

A Closer Look at TCR Germline Recognition

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Over the last 5 years a compendium of structural and functional data on the reactions of T cell receptors (TCRs) that include the germline element Vβ8.2 and other TCR Vβs with related sequences have revealed a very similar interaction motif between residues on the tips of TCR CDR1β, CDR2β, and the α1 helix of the MHC (Figure S1A available online; exemplified in Dai et al., 2008; Feng et al., 2007; Reinherz et al., 1999). The motif includes a trio of amino acids composed of residues Tyr48, Tyr50, and Glu56 (called Tyr46, Tyr48, and Glu54 in papers by some of us) in the CDR2βs of Vβs that are related to Vβ8.2. This has led to the proposal that this conserved motif represents an evolutionary signature of germline specificity. Functionally, mutation of any of these three amino acids in CDR2β usually results in impaired binding to the MHC by TCRs in which the mutant Vβs are included. This is so even in experiments in which the TCRα chain was allowed to adopt any possible naturally generated sequence (Scott-Browne et al., 2009).

In a recent paper published in *Immunity*, Stadinski et al. (2011) isolated TCRs that recognized MHC class II ligands only or cross-reacted with classical and nonclassical MHC class I ligands. These TCRs used the same Vβ8.2 TCR chain (Yae62) paired with different Vα domains and were crystallized in complex with the same class II MHC ligand (Dai et al., 2008; Stadinski et al., 2011). The authors suggest that, by virtue of pairing to an alternative Vα, the TCRβ loop conformations changed and the interaction site of the Vβ domain was significantly modified, leading to an "altered pMHC binding mode" and "modified TCRβ binding reaction with MHC." They go on to conclude that, consequently, germline specificity, defined as conserved pairwise interactions between Vβ and MHC amino acids, is not consistently observed. Indeed, prior examination of the various complexes between TCRs containing Vβ8.2 and MHC revealed that although Y50 of Vβ8.2 (for example) almost always interacts with MHC, it does not do so in each case with the same atom-to-atom contacts (Garcia et al., 2009; Marrack et al., 2008). As such, the idea that TCR chains, in the course of evolution, have evolved germline residues to interact in a consistent way with MHC molecules appears to have less validity. This idea is further emphasized in the accompanying preview by Turner and Rossjohn (2011) who argue that because the differential usage of Vαs "enables the same Vβ chain to recognize a different region of the same MHC," the previous notions of germline specificity based on studies of Vβ8.2 are "inaccurate."

A close examination of the two featured structures and the mutational data in Stadinski et al. (2011) does not lead to all the conclusions drawn. Rather, it provides further evidence for germline specificity. The Vβ footprints of the new structures are very similar to all previous Vβ8.2-I-A complexes. Any of the observed small deviations fall within the range already seen among the previous structures (Figure S1A). Analysis of the degree of overlap between the CDR1β and CDR2β of the two TCRs highlighted in Stadinski et al. (2011) reveals a root mean square deviation (rmsd) for carbon-α atoms of 0.99 Å and 2.0 Å, respectively. By comparison, the rmsd between CDR1β and CDR2β of either Yae62 or J809.B5 and any of the other Vβ8.2-I-A complexes determined so far (and shown in Figure S1) averages approximately 1.15 Å and 2.3 Å rmsd, respectively. Thus, Vβ germline contacts of Yae62 and J809.B5 in fact deviate slightly *less* from one another than from other previously determined Vβ8.2 complexes. Moreover, a closer view of the regions of interest, the CDR1β and CDR2β loop contacts with the α1 helix, shows a very close structural superimposition of not only the main chains but also the side chains mediating the interactions between the structures (Figure S1B).

Certainly there are differences between the Yae62 and J809.B5 structures. For instance, there is an alternative rotamer conformation of MHC residue Gln61 (Figure S1B). Also, there are some small differences in the atom-to-atom engagement of MHC by the trio of Vβ CDR2 amino acids in the Stadinski et al. (2011) structures and those previously described. However, on the whole, the involvement of the Vβ amino acid trio (Y48, Y50, and E56) in engagement of MHCII is very similar in all TCR-MHC II complexes that have been studied to date.

Perhaps our hypothesis about the nature of germline interactions between TCR V regions and MHC has not been stated clearly enough. We did not intend to suggest that an absolutely superimposable atomic identity, with invariant atom-to-atom contacts, would occur in all complexes containing this germline element. Rather, we suggested that the general features of the recognition motif

on the TCRs, principally the location of the contact site and the centrality of the Tyr50 residue (in Vβs that include it) would be preserved (Marrack et al., 2008). Further, our assertion of "pairwise" similarity (Feng et al., 2007) includes the idea that germline specificity "takes two to tango." Thus there must be sites on MHC proteins that are tailored to partner with the MHC-binding motifs on TCRs. We suggest that these general features must have been evolutionarily selected to allow for some flexibility, wobble, or even shifting. This flexibility could encompass different rotamer positions of amino acids within the recognition motif (such as the Vβ-MHC Gln61 changes noted by Stadinski et al. [2011]) that could result in slightly different pairings of specific amino acids. In fact, we venture that this ability of particular TCR amino acids to bind their MHC ligands in a flexible way is essential to the function of the TCR (Marrack et al., 2008). It allows TCRs to have some consistent specificity for MHC while enabling their allotted binding sites to accommodate the demands imposed by differences in length and amino acid composition of TCR CDR3 regions and MHC-engaged peptides.

The results of Stadinski et al. (2011) and others (Dai et al., 2008; Feng et al., 2007; Reinherz et al., 1999) support this idea, as does a paper in the same issue of *Immunity* that describes a strikingly superimposable Vα3-H-2L^d germline interaction motif that is preserved by TCRs bearing different Vβ chains and CDR3 sequences, as well as recognizing entirely distinct peptide antigens through different chemistries (Adams et al., 2011). In fact, in several of the aforementioned Vβ8.2-I-A complexes that contain multiple complexes in the asymmetric unit of the crystal lattice (Reinherz et al., 1999), slight variations in the H-bonding networks of the interaction motif can be seen in the different complexes, indicating once again that there is malleability to the interaction site between TCR and MHC.

Another misunderstood issue involves the idea that multiple modes of germline-encoded interactions probably exist. Thus, a given V region may be able to engage different MHC targets in different ways, but using the same V region amino acids (Garcia et al., 2009). This notion is most dramatically illustrated by the interaction of Vβ8.2-bearing TCRs with the nonclassical MHC protein CD1d. CD1d presents antigens to TCRs that are entirely different from those manifested by classical MHC proteins. Moreover, TCRs bind CD1d in a configuration that is significantly different from that of their engagement of conventional MHC. Nevertheless the Vβ8.2 Tyr50 residue remains key to this interaction (Borg et al., 2007).

Although deviations from (or exceptions to) the codon "rules" can (and will) always be found, such as appears to be the case for super-bulged peptide complexes where the TCR engages the peptide while being held away at some distance from the MHC—much in the manner of an antibody-peptide interaction (Burrows et al., 2010)—we surmise that for the vast majority of TCRs, MHC specificity is germline encoded.

SUPPLEMENTAL INFORMATION

Supplemental Information includes one figure and can be found with this article online at http://dx.doi.org/10.1016/j.immuni.2012.05.018.

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